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A PROGRAM FOR THE STUDY OF SKELETAL MUSCLE CATABOLISM
FOLLOWING PHYSICAL TRAUMA(U) BRIGHAM AND WOMEN'S
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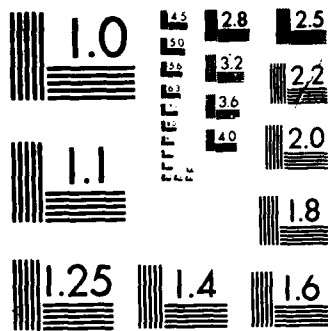
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A PROGRAM FOR THE STUDY OF SKELETAL MUSCLE CATABOLISM
FOLLOWING PHYSICAL TRAUMA

ANNUAL REPORT

February 21, 1986 - February 20, 1987

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Summary

Following laparotomy and retroperitoneal dissection, dogs increase skeletal muscle proteolysis and excrete increased quantities of nitrogen in the urine. Pretreatment with cyclo-oxygenase inhibitor (Ibuprofen) diminished this response in the 5 animals studied to date. More animals are required to make firm conclusions as to the effectiveness of this therapy on post traumatic metabolism.

The metabolic response to injury is characterized by an increased net breakdown of skeletal muscle protein (1). The amino acids released from muscle by this process are transported to other tissues (2), where they provide precursors for the synthesis of glucose and acute phase proteins, serve as fuel for the intestine and participate in wound repair (3). The biochemical processing of amino acid nitrogen results in accelerated formation of urea and ammonia, and these end products of nitrogen metabolism are excreted at increased rates in the urine. Markers which are thought to reflect skeletal muscle proteolysis such as creatine, creatinine, zinc and 3-methyl histidine are also excreted in greater amounts (4,5). In addition the intracellular concentration of free amino acids falls (6). While these catabolic events may have protective and adaptive functions following severe stress, prolonged protein catabolism results in marked protein wasting and concomitant weight loss. Such alterations in body composition lead to fatigue, weakness and eventually are manifest by increased morbidity and mortality.

The specific mediators of these responses are not clearly defined. Neurohormonal mechanisms have been implicated, and inhibition of the usual rise in concentration of "catabolic" hormones such as glucagon, epinephrine, norepinephrine, and cortisol has been shown to block the expected afflux of amino acids from skeletal muscle in the post-operative phase (7). However, this had no effect on the excretion of nitrogenous waste products in the urine. One factor recently implicated as the possible messenger in the catabolic responses is the prostaglandins. It has been shown invitro that an increased rate of proteolysis occurred in isolated rat skeletal muscle when it was incubated with arachidonate, a precursor of prostaglandin and also that inhibitors of prostaglandin synthesis prevented this catabolism (8). Of various prostaglandins studied, it was found that only prostaglandin E2 initiated accelerated protein breakdown. Whether prostaglandins influence post-traumatic protein catabolism invivo is unknown.



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This study investigates the effects of skeletal muscle protein metabolism and urea production of preventing prostaglandin synthesis by cyclo-oxygenase inhibition in animals undergoing a standard abdominal operation known to accelerate protein catabolism.

Five animals underwent the standard operation to serve as controls and another five underwent the same operation while receiving cyclo-oxygenase inhibitor Ibuprofen. As with others in its class, Ibuprofen interferes with the metabolism of arachidonic acid and prevents subsequent production of prostaglandins including prostaglandin E2 (9).

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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Materials and Methods

1) Animal Model

Ten conditioned mongrel dogs were obtained one week prior to study and housed according to the guidelines of the Committee on Animals at the Harvard Medical School and the Committee on the Use of Laboratory Animals of the Institute for Laboratory Animal Resources, the National Research Council of the U.S.A. They were fed standard dry dog food daily (Agway Respond 2000, at least 25% protein by weight), provided water ad libitum, exercised daily and trained to stand quietly in a Pavlov sling. The animals were considered suitable for study if they demonstrated normal food intake, maintenance of the body weight and temperature below 38°C.

At least 3 days prior to operation, arterial whole blood and venous plasma samples were obtained after an overnight fast. A percutaneous muscle biopsy was then obtained during a short general anaesthetic (sodium thiopental, Abbott Laboratories, North Chicago; 5 mg/kg i.v.) and processed using the technique described by Bergstrom (10). The animals were then returned to the kennel to recover from the procedure.

On the night before the operation, all food was removed from the kennel at 5 pm and no food was offered to the animals for the next 48 hours. On the morning of the operation, the animals were exercised and brought to the operating room. A paw vein was cannulated and anesthesia induced with sodium pentobarbital (Abbott Laboratories; 30 mg/kg i.v.). The dogs were intubated and allowed to breath spontaneously. The abdomen and flanks were shaved and prepared in an aseptic fashion and the bladder catheterized to follow continuous collection of urine. A catheter was introduced into the internal jugular vein for the administration of 0.9% saline at 4 mL/hr.kg. The animals underwent a laparotomy via a lower midline incision. After retroperitoneal dissection, catheters were placed in the aorta and vena cava tributaries. The first catheter was positioned with its tip 1 cm above the aortic bifurcation. The second catheter was placed with its tip 1 cm above (cephalad to) the first. The third was placed in the vena cava with its tip below the renal veins. The first catheter was used to infuse para-amino hippuric acid (PAH) at a standard rate so that the blood flow across the hindquarter at any one time could be determined by dye-dilution techniques (11) following measurement of arterial and venous levels of PAH in the second and third catheters. The second and third catheters were also used to measure the arterio-venous difference of amino acids across the hindquarter which represents 50% of the dog's skeletal muscle mass (12).

All three catheters were capped and buried in subcutaneous pockets for subsequent access via a hypodermic needle (11). The

wound was closed and the dogs allowed to recover from the general anesthetic while body temperature was maintained with blankets. At five hours after the start of the operation, the animals were placed in a Pavlov sling and kept there for the remainder of the 24 hour post-operative period. At this same time the infusion of 0.5% PAH was begun at a rate of 0.7 ml/min. This allowed a steady state to be achieved prior to sampling. At six hours after the start of the operation three pairs of arterial and venous blood samples were drawn and processed for amino acid and PAH analysis. The 6 hour time point was chosen because previous studies in our laboratory have shown the rate of release of amino acids from the hindquarter to be maximal at this time (13,14).

The skeletal muscle biopsy was repeated at 24 hours after the start of the operation and processed as before. Again, earlier studies demonstrated that the intracellular concentration of glutamine fell significantly by this time after abdominal surgery in the dog (13,14). The intracellular level of this amino acid has been shown to correlate with the extent of stress in various studies (15,16).

2) Biochemical analysis and calculations

A total of 15 amino acids were measured in three sets of arterial and venous samples at 6 hours post-op for each dog. A mean was calculated from three measurements. This was also the case for the "basal" studies. The micromolar amounts of nitrogen carried by each amino acid were added to obtain a value for total amino-nitrogen. The mean arterio-venous difference was then calculated by simple subtraction. Arterio-venous difference does not give a complete impression of amino acid balance across the hindquarter as it does not take flow into account. If arterio-venous difference is multiplied by flow then a more useful flux is obtained.

Flow was calculated by dye-dilution techniques as explained earlier. At the same time as the amino acid samples were drawn, further samples were drawn over a minute period into heparinized tubes in order to interfere as little as possible with the normal flow through the region. Samples were again drawn in triplicate and a mean value calculated. Flow was calculated from the formula:

$$\text{Flow (L/min)} = \frac{\text{Dye infusion rate (mg/min)}}{\text{Arterial Conc (mg/L)} - \text{Venous Conc (mg/L)}}$$

Total amino-nitrogen concentration in arterial whole blood was calculated for pre-op, 6 hour post-op and basal samples by adding the micromolar nitrogen carried by each amino acid.

Intracellular glutamine concentration in pre- and post-op muscle samples was calculated from enzymatic measurements of glutamine in muscle, whole blood and plasma samples using intracellular water estimates as described by Bergstrom (10).

All urine passed during the 24 hours after the start of the operation was collected and analyzed for total nitrogen, urea and creatinine.

3) Study groups

Five animals were studied as described above and served as controls. Another five dogs underwent the same procedure while receiving the cyclo-oxygenase inhibitor Ibuprofen. The drug was given as an intravenous bolus of 12.5 mg/kg with subsequent doses at three hourly intervals for the next 24 hours. Dogs given this dose regime in another study were found to have serum levels within the therapeutic range throughout the study period (17).

The results were subjected to paired and unpaired student t tests as appropriate.

Results

The operative procedure lasted approximately 2 hours and all animals were extubated and regaining consciousness by 5 hours after induction of general anesthesia. Core temperature was maintained in all animals throughout the study.

The dogs receiving Ibuprofen excreted 332 ± 14 mg/kg body weight of urea (Table 1). This is significantly lower than the value of 409 ± 26 mg/kg found in control animals ($p < 0.05$). This difference could not be accounted for by alterations in the level of blood urea, as there was no net rise in blood urea nitrogen concentration (16.2 ± 0.8 mg/dl pre-op vs. 12.6 ± 4.7 mg/dl post-op). Urinary creatinine excretion was significantly lower in Ibuprofen treated animals than controls (Table 1). Again, this was not due to any net change in plasma creatinine levels. The difference in total nitrogen excretion in urine between the two groups just failed to reach statistical significance ($p = 0.052$). This may be due to a combination of small sample size and some variability in the miscellaneous smaller contributors to total nitrogen excretion which were not all measured in this study.

Arterial amino-nitrogen concentration fell within 6 hours of the operation in both groups (Table 2). This was accounted for in large part by a decline in the concentrations of both alanine and glutamine. The fall in whole blood amino-nitrogen was

significantly less in animals receiving the cyclo-oxygenase inhibitor ($p=0.05$).

Only three values for amino acid efflux are available from animals receiving Ibuprofen. For these three dogs total amino acid release from the hindquarter at 6 hours after operation was $7.6 \pm 0.4 \mu\text{mol}/\text{min}.\text{kg}$. While it is not appropriate to apply statistical analysis to such small numbers, this would appear to be lower than the value of $19.1 \pm 4.1 \mu\text{mol}/\text{min}.\text{kg}$ obtained in the control post-operative group (Table 2). This trend could not be accounted for by changes in bloodflow, and it was therefore due to a general reduction in the rate of release of most of the amino acids measured. Of particular interest is the apparent reduction in the efflux of phenylalanine which has been shown to be a marker of skeletal muscle breakdown (18).

The intracellular concentration of the amino acid glutamine in muscle fell to a similar extent in both groups by 24 hours after operation (from 21.2 ± 2.6 to $16.7 \pm 3.2 \text{ mmol/L}$ in controls and from 25.6 ± 0.9 to $20.2 \pm 1.3 \text{ mmol/L}$ in ibuprofen treated dogs).

Discussion

The surgical model used in this study produces several changes in protein catabolism which are consistent with mild to modest injury. These include an increase in the urinary excretion of urea and creatinine, net release of amino acids from the hindquarter, reduced whole blood amino acid nitrogen and decreased intracellular glutamine in skeletal muscle. Accelerated skeletal muscle protein catabolism in humans following injury similarly has been associated with increased amino acid release from skeletal muscle (2), a fall in intravenous cellular glutamine concentration (6) and increased excretion of creatinine in the urine (4).

It appears that there is an attenuation of the post traumatic response with Ibuprofen treatment. More animals are needed to confirm these preliminary findings.

Table 1

Urinary excretion of nitrogen, urea and creatinine in dogs receiving Ibuprofen peri-operatively and controls (\pm SEM)

	N	Dog Weight (kg)	Urine Volume (mg/24hr.kg)	Total Nitrogen (mg/24hr.kg)	Urea (mg/24hr.kg)	Creatinine (mg/24hr.kg)
Control	5	26.8 \pm 2.2	39.4 \pm 5.5	492 \pm 20	409 \pm 26	39 \pm 2
Ibuprofen	5	23.2 \pm 0.8	35.6 \pm 4.0	445 \pm 23	332 \pm 14*	27 \pm 3*

*Different from control ($p < 0.05$)

Table 2

Amino acid concentration in arterial whole blood and afflux of amino nitrogen and phenyl alanine from hindquarter muscle in basal animals, post-op controls and Ibuprofen-treated post-op dogs (\pm SEM)

	Whole Blood amino acid nitrogen (μ mol/L)	Hindquarter afflux	
		Amino acid nitrogen (μ mol/min.kg)	Phenyl alanine (μ mol/min.kg)
Basal	4715 \pm 295	0.5 \pm 5.3	0.24 \pm 0.19
Control Post-op	3733 \pm 106*	19.1 \pm 4.1*	0.34 \pm 0.05
Ibuprofen Post-op	4159 \pm 223**	7.6 \pm 0.4	0.18 \pm 0.06

*Different from basal ($p < 0.05$)

**Different from post-op control ($p < 0.05$)

Table 3

Afflux of amino-nitrogen and phenyl alanine from hindquarter
at 6 hours post-op with different forms of blockade (\pm SEM)

	Amino-nitrogen afflux (μ mol/min.kg)	Phenylalanine afflux (μ mol/min.kg)
Control	19.1 \pm 4.1	0.34 \pm 0.05
Adrenergic blockade	6.9 \pm 1.2*	0.16 \pm 0.03*
Epidural blockade	9.0 \pm 0.9*	0.24 \pm 0.02*
Prostaglandin	7.6 \pm 0.4	0.18 \pm 0.06

*Significantly different from control ($p < 0.05$)

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Personnel Receiving Contract Support

Individuals working on this project, who received contract support are:

	Percentage of salary
Douglas Wilmore	10%
Hamish Michie	100%
Ramona Faris	50%

Addendum to Annual Report

Prepared August 5, 1987

The following comments acknowledge the corrections/additions which have been made on the original document which was received in our office July 21, 1987. These changes have been incorporated in the body of this final report. The following additions are included in this report to clarify issues which were raised in the review of the annual report.

Major concerns raised:

1. Progress on this contract is inadequate. This project was submitted to the Army for review in September 1984 with a proposed starting date of March 16, 1985. The review process and final contract processing was not finalized until February 1986. This was not a continuation of an old contract but the start of a new contract. Because of the time lapse between submission and initiation of this project, personnel changes occurred. New individuals needed to be recruited and trained to perform the work. These personnel changes were on record at this institution at the time of initiation of this contract and were reviewed with Dr Wade during his visit to this facility on January 28, 1987. The current individuals on this contract are:

Name	% effort
Douglas Wilmore	10
Hamish Mitchie	100
Romonna Faris	50

In order to insure high quality scientific work, preliminary studies were performed during the initiation of the contract. In addition to the results reported, four hindleg infusion experiments were performed during this report period (Feb 21, 1986-Feb 20, 1987) and these investigations have continued through the spring of 1987.

This model is described in detail in the contract application. Briefly, the dog is anesthetized and both hind limbs are catheterized. Blood flow is determined using dye dilution technique. Substrate flux is measured from the legs periodically over the 6 hour study period and flux calculated. A portion of the data is shown:

TIME = 0		TIME = 6 hrs	
Right Leg	Left leg	Right Leg	Left Leg
Blood Flow (ml/min.kg)			
6.49	5.96	4.80	6.32
±1.09	±1.77	±0.41	±0.78
Glucose Exchange (mg/min.kg)			
0.10	0.15	0.01	0.06
±0.06	±0.10	±0.12	±0.20
Alamine Plus Glutamine Nitrogen Exchange (μmol/min.kg)			
-0.020	-0.146	-0.267	-0.380
±0.160	±0.138	±0.057	±0.058

(by convention - = release and + = uptake)

These data show reasonable comparability between both limbs and stabilization of flow over time. It appears that there is a gradual decrease in glucose uptake over time and increase in amino acid release, but there is not sufficient data available to statically confirm this trend.

Thus, the work in the first year has been gradual because of the reorganization and training of personnel and the use of quality control measures to insure high quality scientific work.

2. Twenty-five studies were to be conducted in the first year. In addition to the gradual start up as described above, there has been a major increase in the price of animals and animal board during the first year of the study. Over the past year the cost of dog purchase has risen to \$400/animal (from \$215) with the cost of board now \$8.04/day (from \$4.80). At these costs, we would require an additional 5-6000 /year in order to complete this number of studies. In addition, it is anticipated that animal costs will rise an additional 10% in the coming year. These costs changes were reviewed with Dr. Wade during his January site visit. If significance of the data can be obtained using fewer animals, this course will be followed rather than request additional funds.

3. Historical controls were used for the control data. The review is correct that historical controls were referred to in the report. Since the report was submitted we have initiated a series of paired studies to evaluate the effect of ibuprofen in the post operative dog which follows the protocol as outlined on page 22 of the proposed contract. As noted on page 8 of the annual report, these studies should be regarded as preliminary findings until these investigations are complete.

In summary, the year covered by this annual report represented a start-up period in which new personnel were placed on the project and they learned new techniques. In addition, costs for animals and housing have increased markedly. We have faced these realities and used our best efforts to initiate high quality scientific work on this project.

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